=> d his

(FILE 'HOME' ENTERED AT 16:23:35 ON 06 JUN 2002)

INDEX 'ADISALERTS, ADISINSIGHT, ADISNEWS, AGRICOLA, ANABSTR, AQUASCI, BIOBUSINESS, BIOCOMMERCE, BIOSIS, BIOTECHABS, BIOTECHDS, BIOTECHNO, CABA, CANCERLIT, CAPLUS, CEABA-VTB, CEN, CIN, CONFSCI, CROPB, CROPU, DDFB, DDFU, DGENE, DRUGB, DRUGLAUNCH, DRUGMONOG2, ...' ENTERED AT 16:24:09 ON 06 JUN 2002

SEA RNAS? AND (DNA? OR PLASMI?)

---

8 FILE ADISALERTS
9 FILE ADISINSIGHT
844 FILE AGRICOLA
19 FILE ANABSTR
231 FILE AQUASCI
108 FILE BIOBUSINESS
43 FILE BIOCOMMERCE
10723 FILE BIOSIS
463 FILE BIOTECHABS
463 FILE BIOTECHDS
6946 FILE BIOTECHNO
1779 FILE CABA
4020 FILE CANCERLIT
14914 FILE CAPLUS
103 FILE CEABA-VTB
32 FILE CEN
22 FILE CIN
19 FILE CONFSCI
2 FILE CROPB
25 FILE CROPU
4 FILE DDFB
123 FILE DDFU
39403 FILE DGENE
4 FILE DRUGB
7 FILE DRUGNL
260 FILE DRUGU
6 FILE DRUGUPDATES
44 FILE EMBAL
8355 FILE EMBASE
3511 FILE ESBIOBASE
548 FILE FEDRIP
9 FILE FROSTI
68 FILE FSTA
195265 FILE GENBANK
463 FILE IFIPAT
524 FILE JICST-EPLUS
3 FILE KOSMET
4474 FILE LIFESCI
11798 FILE MEDLINE
33 FILE NIOSHTIC
99 FILE NTIS
38 FILE OCEAN
1644 FILE PASCAL
16 FILE PHAR
1 FILE PHIC
18 FILE PHIN
265 FILE PROMT
8607 FILE SCISEARCH
3279 FILE TOXCENTER
14388 FILE USPATFULL
52 FILE USPAT2
590 FILE WPIDS
590 FILE WPINDEX
8 FILE NAPRALERT
429 FILE NLDB

L1 QUE RNAS? AND (DNA? OR PLASMI?)

FILE 'GENBANK, DGENE, CAPLUS, USPATFULL, MEDLINE, BIOSIS, SCISEARCH, EMBASE, BIOTECHNO, LIFESCI, CANCERLIT, ESBIOBASE, TOXCENTER, CABA, PASCAL, AGRICOLA, WPIDS, FEDRIP, JICST-EPLUS' ENTERED AT 16:26:32 ON 06 JUN 2002

L2 290661 S RNAS? (S) (DNA? OR PLASMI?)
L3 583 S L2 (S) (CELL? (A) LYS?)
L4 359 DUP REM L3 (224 DUPLICATES REMOVED)
L5 359 FOCUS L4 1-
L6 87836 S RNAS? (S) (CLON? OR EXPRESS?) (S) (BACTERI? OR COLI?)
L7 59604 S L6 (S) (GEN?)
L8 23 S L7 (S) SECRET? (S) PERIPLASM?
L9 11 DUP REM L8 (12 DUPLICATES REMOVED)

```
Welcome to STN International!  Enter x:x

LOGINID:ssspta1652dmr

PASSWORD:
TERMINAL (ENTER 1, 2, 3, OR ?):2


* * * * * * * * * *   Welcome to STN International   * * * * * * * * * *

 NEWS  1           Web Page URLs for STN Seminar Schedule - N. America
 NEWS  2  Jan 25   BLAST(R) searching in REGISTRY available in STN on the Web
 NEWS  3  Jan 29   FSTA has been reloaded and moves to weekly updates
 NEWS  4  Feb 01   DKILIT now produced by FIZ Karlsruhe and has a new update
                   frequency
 NEWS  5  Feb 19   Access via Tymnet and SprintNet Eliminated Effective 3/31/02
 NEWS  6  Mar 08   Gene Names now available in BIOSIS
 NEWS  7  Mar 22   TOXLIT no longer available
 NEWS  8  Mar 22   TRCTHERMO no longer available
 NEWS  9  Mar 28   US Provisional Priorities searched with P in CA/CAplus
                   and USPATFULL
 NEWS 10  Mar 28   LIPINSKI/CALC added for property searching in REGISTRY
 NEWS 11  Apr 02   PAPERCHEM no longer available on STN.  Use PAPERCHEM2 instead.
 NEWS 12  Apr 08   "Ask CAS" for self-help around the clock
 NEWS 13  Apr 09   BEILSTEIN: Reload and Implementation of a New Subject Area
 NEWS 14  Apr 09   ZDB will be removed from STN
 NEWS 15  Apr 19   US Patent Applications available in IFICDB, IFIPAT, and IFIUDB
 NEWS 16  Apr 22   Records from IP.com available in CAPLUS, HCAPLUS, and ZCAPLUS
 NEWS 17  Apr 22   BIOSIS Gene Names now available in TOXCENTER
 NEWS 18  Apr 22   Federal Research in Progress (FEDRIP) now available
 NEWS 19  May 31   PCTFULL to be reloaded.  File temporarily unavailable.
 NEWS 20  Jun 03   New e-mail delivery for search results now available

 NEWS EXPRESS  February 1 CURRENT WINDOWS VERSION IS V6.0d,
               CURRENT MACINTOSH VERSION IS V6.0a(ENG) AND V6.0Ja(JP),
               AND CURRENT DISCOVER FILE IS DATED 05 FEBRUARY 2002
 NEWS HOURS    STN Operating Hours Plus Help Desk Availability
 NEWS INTER    General Internet Information
 NEWS LOGIN    Welcome Banner and News Items
 NEWS PHONE    Direct Dial and Telecommunication Network Access to STN
 NEWS WWW      CAS World Wide Web Site (general information)

Enter NEWS followed by the item number or name to see news on that
specific topic.

  All use of STN is subject to the provisions of the STN Customer
  agreement.  Please note that this agreement limits use to scientific
  research.  Use for software development or design or implementation
  of commercial gateways or other similar uses is prohibited and may
  result in loss of user privileges and other penalties.

  * * * * * * * * * * * * * * STN  Columbus  * * * * * * * * * * * * * * *



FILE 'HOME' ENTERED AT 16:23:35 ON 06 JUN 2002

=> index bioscience medicine
FILE 'DRUGMONOG' ACCESS NOT AUTHORIZED
COST IN U.S. DOLLARS                                SINCE FILE      TOTAL
                                                       ENTRY    SESSION
FULL ESTIMATED COST                                     0.21       0.21
```

```
INDEX 'ADISALERTS, ADISINSIGHT, ADISNEWS, AGRICOLA, ANABSTR, AQUASCI,
      BIOBUSINESS, BIOCOMMERCE, BIOSIS, BIOTECHABS, BIOTECHDS, BIOTECHNO, CABA,
      CANCERLIT, CAPLUS, CEABA-VTB, CEN, CIN, CONFSCI, CROPB, CROPU, DDFB,
      DDFU, DGENE, DRUGB, DRUGLAUNCH, DRUGMONOG2, ...'
ENTERED AT 16:24:09 ON 06 JUN 2002

64 FILES IN THE FILE LIST IN STNINDEX

Enter SET DETAIL ON to see search term postings or to view
search error messages that display as 0* with SET DETAIL OFF.

=> s rnas? and (dna? or plasmi?)
          8   FILE ADISALERTS
          9   FILE ADISINSIGHT
        844   FILE AGRICOLA
         19   FILE ANABSTR
        231   FILE AQUASCI
        108   FILE BIOBUSINESS
         43   FILE BIOCOMMERCE
      10723   FILE BIOSIS
        463   FILE BIOTECHABS
        463   FILE BIOTECHDS
       6946   FILE BIOTECHNO
       1779   FILE CABA
       4020   FILE CANCERLIT
      14914   FILE CAPLUS
        103   FILE CEABA-VTB
         32   FILE CEN
         22   FILE CIN
         19   FILE CONFSCI
          2   FILE CROPB
         25   FILE CROPU
  21 FILES SEARCHED...
          4   FILE DDFB
        123   FILE DDFU
      39403   FILE DGENE
          4   FILE DRUGB
          7   FILE DRUGNL
        260   FILE DRUGU
          6   FILE DRUGUPDATES
         44   FILE EMBAL
       8355   FILE EMBASE
       3511   FILE ESBIOBASE
        548   FILE FEDRIP
  36 FILES SEARCHED...
          9   FILE FROSTI
         68   FILE FSTA
     195265   FILE GENBANK
        463   FILE IFIPAT
        524   FILE JICST-EPLUS
          3   FILE KOSMET
       4474   FILE LIFESCI
      11798   FILE MEDLINE
         33   FILE NIOSHTIC
         99   FILE NTIS
         38   FILE OCEAN
       1644   FILE PASCAL
  50 FILES SEARCHED...
         16   FILE PHAR
          1   FILE PHIC
         18   FILE PHIN
        265   FILE PROMT
       8607   FILE SCISEARCH
       3279   FILE TOXCENTER
```

```
      14388   FILE USPATFULL
         52   FILE USPAT2
        590   FILE WPIDS
        590   FILE WPINDEX
          8   FILE NAPRALERT
        429   FILE NLDB

  55 FILES HAVE ONE OR MORE ANSWERS,   64 FILES SEARCHED IN STNINDEX

L1   QUE RNAS? AND (DNA? OR PLASMI?)

=> d rank
F1       195265   GENBANK
F2        39403   DGENE
F3        14914   CAPLUS
F4        14388   USPATFULL
F5        11798   MEDLINE
F6        10723   BIOSIS
F7         8607   SCISEARCH
F8         8355   EMBASE
F9         6946   BIOTECHNO
F10        4474   LIFESCI
F11        4020   CANCERLIT
F12        3511   ESBIOBASE
F13        3279   TOXCENTER
F14        1779   CABA
F15        1644   PASCAL
F16         844   AGRICOLA
F17         590   WPIDS
F18         590   WPINDEX
F19         548   FEDRIP
F20         524   JICST-EPLUS
F21         463   BIOTECHABS
F22         463   BIOTECHDS
F23         463   IFIPAT
F24         429   NLDB
F25         265   PROMT
F26         260   DRUGU
F27         231   AQUASCI
F28         123   DDFU
F29         108   BIOBUSINESS
F30         103   CEABA-VTB
F31          99   NTIS
F32          68   FSTA
F33          52   USPAT2
F34          44   EMBAL
F35          43   BIOCOMMERCE
F36          38   OCEAN
F37          33   NIOSHTIC
F38          32   CEN
F39          25   CROPU
F40          22   CIN
F41          19   ANABSTR
F42          19   CONFSCI
F43          18   PHIN
F44          16   PHAR
F45           9   ADISINSIGHT
F46           9   FROSTI
F47           8   ADISALERTS
F48           8   NAPRALERT
F49           7   DRUGNL
F50           6   DRUGUPDATES
F51           4   DDFB
F52           4   DRUGB
F53           3   KOSMET
```

```
F54             2   CROPB
F55             1   PHIC


=> file f1-f20
COST IN U.S. DOLLARS                                SINCE FILE      TOTAL
                                                         ENTRY    SESSION
FULL ESTIMATED COST                                       2.12       2.33

FILE 'GENBANK' ENTERED AT 16:26:32 ON 06 JUN 2002

FILE 'DGENE' ENTERED AT 16:26:32 ON 06 JUN 2002
COPYRIGHT (C) 2002 THOMSON DERWENT

FILE 'CAPLUS' ENTERED AT 16:26:32 ON 06 JUN 2002
USE IS SUBJECT TO THE TERMS OF YOUR STN CUSTOMER AGREEMENT.
PLEASE SEE "HELP USAGETERMS" FOR DETAILS.
COPYRIGHT (C) 2002 AMERICAN CHEMICAL SOCIETY (ACS)

FILE 'USPATFULL' ENTERED AT 16:26:32 ON 06 JUN 2002
CA INDEXING COPYRIGHT (C) 2002 AMERICAN CHEMICAL SOCIETY (ACS)

FILE 'MEDLINE' ENTERED AT 16:26:32 ON 06 JUN 2002

FILE 'BIOSIS' ENTERED AT 16:26:32 ON 06 JUN 2002
COPYRIGHT (C) 2002 BIOLOGICAL ABSTRACTS INC.(R)

FILE 'SCISEARCH' ENTERED AT 16:26:32 ON 06 JUN 2002
COPYRIGHT (C) 2002 Institute for Scientific Information (ISI) (R)

FILE 'EMBASE' ENTERED AT 16:26:32 ON 06 JUN 2002
COPYRIGHT (C) 2002 Elsevier Science B.V.  All rights reserved.

FILE 'BIOTECHNO' ENTERED AT 16:26:32 ON 06 JUN 2002
COPYRIGHT (C) 2002 Elsevier Science B.V., Amsterdam. All rights reserved.

FILE 'LIFESCI' ENTERED AT 16:26:32 ON 06 JUN 2002
COPYRIGHT (C) 2002 Cambridge Scientific Abstracts (CSA)


FILE 'CANCERLIT' ENTERED AT 16:26:32 ON 06 JUN 2002

FILE 'ESBIOBASE' ENTERED AT 16:26:32 ON 06 JUN 2002
COPYRIGHT (C) 2002 Elsevier Science B.V., Amsterdam. All rights reserved.

FILE 'TOXCENTER' ENTERED AT 16:26:32 ON 06 JUN 2002
COPYRIGHT (C) 2002 ACS

FILE 'CABA' ENTERED AT 16:26:32 ON 06 JUN 2002
COPYRIGHT (C) 2002 CAB INTERNATIONAL (CABI)

FILE 'PASCAL' ENTERED AT 16:26:32 ON 06 JUN 2002
Any reproduction or dissemination in part or in full,
by means of any process and on any support whatsoever
is prohibited without the prior written agreement of INIST-CNRS.
COPYRIGHT (C) 2002 INIST-CNRS. All rights reserved.

FILE 'AGRICOLA' ENTERED AT 16:26:32 ON 06 JUN 2002

FILE 'WPIDS' ENTERED AT 16:26:32 ON 06 JUN 2002
COPYRIGHT (C) 2002 THOMSON DERWENT

FILE 'WPINDEX' ACCESS NOT AUTHORIZED

FILE 'FEDRIP' ENTERED AT 16:26:32 ON 06 JUN 2002
```

```
FILE 'JICST-EPLUS' ENTERED AT 16:26:32 ON 06 JUN 2002
COPYRIGHT (C) 2002 Japan Science and Technology Corporation (JST)

=> s rnas? (s) (dna? or plasmi?)
   1 FILES SEARCHED...
   8 FILES SEARCHED...
  15 FILES SEARCHED...
PROXIMITY OPERATOR LEVEL NOT CONSISTENT WITH
FIELD CODE - 'AND' OPERATOR ASSUMED 'RNAS? (S) '
L2      290661 RNAS? (S) (DNA? OR PLASMI?)

=> s l2 (s) (cell? (a) lys?)
   2 FILES SEARCHED...
   6 FILES SEARCHED...
   9 FILES SEARCHED...
  11 FILES SEARCHED...
  13 FILES SEARCHED...
  17 FILES SEARCHED...
PROXIMITY OPERATOR LEVEL NOT CONSISTENT WITH
FIELD CODE - 'AND' OPERATOR ASSUMED 'L19 (S) '
L3          583 L2 (S) (CELL? (A) LYS?)

=> dup rem l3
DUPLICATE IS NOT AVAILABLE IN 'GENBANK, DGENE, FEDRIP'.
ANSWERS FROM THESE FILES WILL BE CONSIDERED UNIQUE
PROCESSING COMPLETED FOR L3
L4           359 DUP REM L3 (224 DUPLICATES REMOVED)

=> focus l4
FOCUS NOT AVAILABLE IN 'GENBANK'.
PROCESSING COMPLETED FOR L4
ANSWERS FROM NON FOCUS FILES PUT AT END OF ANSWER SET.
L5           359 FOCUS L4 1-

=> d ti l5 1-100

L5   ANSWER 1 OF 359  CAPLUS  COPYRIGHT 2002 ACS
TI   The use of single-stranded DNA and RNase H to promote
     quantitative 'hybrid arrest of translation' of mRNA/DNA hybrids
     in reticulocyte lysate cell-free translations

L5   ANSWER 2 OF 359  CAPLUS  COPYRIGHT 2002 ACS
TI   Specific proteins of microorganisms as regulators of enzyme activity

L5   ANSWER 3 OF 359  USPATFULL
TI     Process and a device for the isolation of cell components such as
       nucleic acids from natural sources

L5   ANSWER 4 OF 359  USPATFULL
TI     Process and a device for the isolation of cell components such as
       nucleic acids from natural sources

L5   ANSWER 5 OF 359  CAPLUS  COPYRIGHT 2002 ACS
TI   Activation of antigen-specific cytotoxic T lymphocyte by dendritic cells

L5   ANSWER 6 OF 359  CAPLUS  COPYRIGHT 2002 ACS
TI   A rapid microscale technique for isolation of recombinant plasmid DNA
     suitable for restriction enzyme analysis

L5   ANSWER 7 OF 359  CAPLUS  COPYRIGHT 2002 ACS
TI   Autoreactive IgG to intracellular proteins in sera of MS patients

L5   ANSWER 8 OF 359  CAPLUS  COPYRIGHT 2002 ACS
TI   A rapid method for purifying bacterial deoxyribonucleic acid
```

TI Preparation of mesophilic microorganisms which contain a D-hydantoinase which is active at elevated temperature

L5 ANSWER 351 OF 359 USPATFULL
TI Recombinant deoxyribonucleic acid which codes for plasminogen activator and method of making plasminogen activator protein therefrom

L5 ANSWER 352 OF 359 USPATFULL
TI Production of aryl acylamidases

L5 ANSWER 353 OF 359 USPATFULL
TI Recombinant deoxyribonucleic acid which codes for plasminogen activator

L5 ANSWER 354 OF 359 FEDRIP COPYRIGHT 2002 NTIS
TI PURCHASE OF A 600 MHZ NMR SPECTROMETER

L5 ANSWER 355 OF 359 GENBANK.RTM. COPYRIGHT 2002

TITLE (TI): Complete genome sequence of Clostridium perfringens, an anaerobic flesh-eater
TITLE (TI): Direct Submission

L5 ANSWER 356 OF 359 GENBANK.RTM. COPYRIGHT 2002

TITLE (TI): Complete genome sequence of a multiple drug resistant Salmonella enterica serovar Typhi CT18
TITLE (TI): Direct Submission

L5 ANSWER 357 OF 359 GENBANK.RTM. COPYRIGHT 2002

TITLE (TI): Genome sequence of Yersinia pestis, the causative agent of plague
TITLE (TI): Direct Submission

L5 ANSWER 358 OF 359 GENBANK.RTM. COPYRIGHT 2002

TITLE (TI): Complete DNA sequence of a serogroup A strain of Neisseria meningitidis Z2491
TITLE (TI): Direct Submission

L5 ANSWER 359 OF 359 GENBANK.RTM. COPYRIGHT 2002

TITLE (TI): Complete DNA sequence of a serogroup A strain of Neisseria meningitidis Z2491
TITLE (TI): Direct Submission

=> d ti l5 4 6 27 33 36 37 41 47 48 49 58 65 93 116 139 141 237

L5 ANSWER 4 OF 359 USPATFULL
TI Process and a device for the isolation of cell components such as nucleic acids from natural sources

L5 ANSWER 6 OF 359 CAPLUS COPYRIGHT 2002 ACS
TI A rapid microscale technique for isolation of recombinant plasmid DNA suitable for restriction enzyme analysis

L5 ANSWER 27 OF 359 MEDLINE
TI Rapid purification of double-stranded DNA by triple-helix-mediated affinity capture.

L5 ANSWER 33 OF 359 DGENE (C) 2002 THOMSON DERWENT
TI Production of RNA-free cellular components e.g. DNA, proteins, or carbohydrates -

L5 ANSWER 36 OF 359 BIOSIS COPYRIGHT 2002 BIOLOGICAL ABSTRACTS INC.
TI CO ISOLATION OF IN-VIVO PHOSPHORUS-32 LABELED SPECIFIC TRANSCRIPTS AND DNA WITHOUT PHENOL EXTRACTION OR NUCLEASE DIGESTION.

L5 ANSWER 37 OF 359 CAPLUS COPYRIGHT 2002 ACS
TI Effect of ribonuclease on the association of deoxyribonucleic acid with the membrane in Escherichia coli

L5 ANSWER 41 OF 359 LIFESCI COPYRIGHT 2002 CSA
TI A rapid method for the analysis of plasmid content and copy number in various streptomycetes grown on agar plates.

L5 ANSWER 47 OF 359 MEDLINE
TI Purification of essentially RNA free plasmid DNA using a modified Escherichia coli host strain expressing ribonuclease A.

L5 ANSWER 48 OF 359 WPIDS (C) 2002 THOMSON DERWENT
TI Recombinant prodn. of reverse transcriptase in RNase-deficient cells - and subsequent purificn. by cation-exchange chromatography to produce enzyme with low levels of contaminants.

L5 ANSWER 49 OF 359 CAPLUS COPYRIGHT 2002 ACS
TI Rapid isolation and sequencing of double-stranded plasmid DNA

L5 ANSWER 58 OF 359 DGENE (C) 2002 THOMSON DERWENT
TI Production of RNA-free cellular components e.g. DNA, proteins, or carbohydrates -

L5 ANSWER 65 OF 359 USPATFULL
TI Method for large scale plasmid purification

L5 ANSWER 93 OF 359 USPATFULL
TI Method for purifying nucleic acids from heterogenous mixtures

L5 ANSWER 116 OF 359 WPIDS (C) 2002 THOMSON DERWENT
TI Production of RNA-free cellular components e.g. DNA, proteins, or carbohydrates.

L5 ANSWER 139 OF 359 USPATFULL
TI Process for the preparation of endotoxin-free or endotoxin-depleted nucleic acids and/or oligonucleotides for gene therapy

L5 ANSWER 141 OF 359 USPATFULL
TI Process for the separation and purification of nucleic acids from biological sources

L5 ANSWER 237 OF 359 USPATFULL
TI Release of intracellular material and the production therefrom of single stranded nucleic acid

=> d ibib abs l5 4 6 27 33 36 37 41 47 48 49 58 65 93 116 139 141 237

L5 ANSWER 4 OF 359 USPATFULL
ACCESSION NUMBER: 2001:136453 USPATFULL
TITLE: Process and a device for the isolation of cell components such as nucleic acids from natural sources
INVENTOR(S): Colpan, Metin, Essen-Kettwig, Germany, Federal Republic of
PATENT ASSIGNEE(S): Qiagen GmbH, Hilden, Germany, Federal Republic of (non-U.S. corporation)

	NUMBER	KIND	DATE
PATENT INFORMATION:	US 6277648	B1	20010821

APPLICATION INFO.: US 1994-253152 19940602 (8)
RELATED APPLN. INFO.: Continuation-in-part of Ser. No. WO 1992-EP2774, filed on 1 Dec 1992

	NUMBER	DATE
PRIORITY INFORMATION:	DE 1991-4139664	19911202

DOCUMENT TYPE: Utility
FILE SEGMENT: GRANTED
PRIMARY EXAMINER: Kim, John
LEGAL REPRESENTATIVE: Jacobson, Price, Holman & Stern, PLLC
NUMBER OF CLAIMS: 10
EXEMPLARY CLAIM: 1
NUMBER OF DRAWINGS: 10 Drawing Figure(s); 7 Drawing Page(s)
LINE COUNT: 583
CAS INDEXING IS AVAILABLE FOR THIS PATENT.

AB Described is a method of isolating cell components, such as nucleic acids, from natural sources by filtering a sample of the digested natural sources such as cells or cell fragments. The method is characterized in that the sample is passed through a filter, the pore size of which decreases in the direction of flow of the sample through the filter.

CAS INDEXING IS AVAILABLE FOR THIS PATENT.

L5 ANSWER 6 OF 359 CAPLUS COPYRIGHT 2002 ACS
ACCESSION NUMBER: 1980:491453 CAPLUS
DOCUMENT NUMBER: 93:91453
TITLE: A rapid microscale technique for isolation of recombinant plasmid DNA suitable for restriction enzyme analysis
AUTHOR(S): Klein, Ronald D.; Selsing, Erik; Wells, Robert D.
CORPORATE SOURCE: Coll. Agric. Life Sci., Univ. Wisconsin, Madison, WI, 53706, USA
SOURCE: Plasmid (1980), 3(1), 88-91
CODEN: PLSMDX; ISSN: 0147-619X
DOCUMENT TYPE: Journal
LANGUAGE: English

AB A simple and rapid microscale technique is described for the isolation of **plasmid DNA** which involves **cell lysis** with PhOH, centrifugation, PhOH extn., EtOH pptn., and **RNase** digestion. The plasmid DNA is of suitable purity and quantity for multiple restriction endonuclease digestions and bacterial transformations. This miniprep procedure is applicable for a variety of types of plasmids ranging in size from 2900 to 18,400 base pairs (bp) and for a no. of Escherichia coli strains. The plasmids are rapidly cleaved by all restriction enzymes (total of 14 tested). Recombinant clones have been screened for insertions .gtoreq.10 bp and .ltoreq.5000 bp. The procedure takes .apprx.3 h and has been routinely used to simultaneously analyze 24 candidate clones. This procedure is reliable and useful for rapid screening of recombinant DNA candidates where anal. by restriction endonuclease digestion is necessary.

L5 ANSWER 27 OF 359 MEDLINE
ACCESSION NUMBER: 93297739 MEDLINE
DOCUMENT NUMBER: 93297739 PubMed ID: 8517544
TITLE: Rapid purification of double-stranded DNA by triple-helix-mediated affinity capture.
AUTHOR: Ji H; Smith L M
CORPORATE SOURCE: Department of Chemistry, University of Wisconsin-Madison 53706.
SOURCE: ANALYTICAL CHEMISTRY, (1993 May 15) 65 (10) 1323-8.
Journal code: 4NR; 0370536. ISSN: 0003-2700.
PUB. COUNTRY: United States
Journal; Article; (JOURNAL ARTICLE)

LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 199307
ENTRY DATE: Entered STN: 19930806
Last Updated on STN: 19980206
Entered Medline: 19930716

AB A simple and rapid method for the preparation of highly pure **plasmid DNA** has been developed. The **DNA** is directly captured from bacterial **cell lysates** by formation of a triple-helical structure between the **plasmid** dsDNA and a 20-base biotinylated oligonucleotide attached to streptavidin-coated magnetic beads and then eluted from the beads in pH 9 buffer solution. No phenol extraction, ethanol precipitation, **RNase** digestion, or CsCl gradient centrifugation is required. A general purpose cloning vector, pHJ19, was constructed for this application from pUC19 **DNA** by insertion of a 40-base sequence suitable for triple-helix formation. The approach was also found suitable for the purification of lambda bacteriophage **DNA**.

L5 ANSWER 33 OF 359 DGENE (C) 2002 THOMSON DERWENT
ACCESSION NUMBER: AAZ32515 DNA DGENE
TITLE: Production of RNA-free cellular components e.g. DNA, proteins, or carbohydrates -
INVENTOR: Hanak J A J; Williams S G
PATENT ASSIGNEE: (COBR-N) COBRA THERAPEUTICS LTD.
PATENT INFO: WO 9953018 A2 19991021 91p
APPLICATION INFO: WO 1999-GB1124 19990413
PRIORITY INFO: GB 1998-7922 19980414
US 1998-81726 19980414
GB 1998-17151 19980806
DOCUMENT TYPE: Patent
LANGUAGE: English
OTHER SOURCE: 1999-620408 [53]

AN AAZ32515 DNA DGENE

AB PCR primers AAZ32514-Z32515 are used to amplify the **RNase** I gene from the cloning vector pCR3.1RNase1. The PCR product is used in the production of a **plasmid** containing the **RNase** I gene under the control of the lacZ promoter. The **plasmid** is used in the method of the invention which relates to the production of a substantially RNA-free cellular component. The methods involve culturing a cell producing the cellular component and lysing the cells to produce a **cell lysate**, where the lysate contains the cellular component and sufficient **RNase** activity to degrade substantially all of the RNA molecules present in the lysate. The methods can be used for RNA-free production of cellular components, such as **DNA**, proteins, or carbohydrates, which may have commercial or therapeutic value. RNA is a major contaminant of preparations from **cell lysates** that is difficult to remove as it is similar in size and charge to **DNA**. The invention helps to overcome the limitations of previous methods for RNA removal.

L5 ANSWER 36 OF 359 BIOSIS COPYRIGHT 2002 BIOLOGICAL ABSTRACTS INC.
ACCESSION NUMBER: 1982:225851 BIOSIS
DOCUMENT NUMBER: BA73:85835
TITLE: CO ISOLATION OF IN-VIVO PHOSPHORUS-32 LABELED SPECIFIC TRANSCRIPTS AND DNA WITHOUT PHENOL EXTRACTION OR NUCLEASE DIGESTION.
AUTHOR(S): HAYES S; HAYES C; BRAND L
CORPORATE SOURCE: DEPARTMENT OF MICROBIOLOGY, SCHOOL OF MEDICINE, UNIVERSITY OF SASKATCHEWAN, SASKATOON, SASKATCHEWAN, S7N OWO, CANADA.
SOURCE: ANAL BIOCHEM, (1981) 116 (2), 480-488.
CODEN: ANBCA2. ISSN: 0003-2697.
FILE SEGMENT: BA; OLD
LANGUAGE: English

AB A method is described for isolation and quantitation of specific intact

transcripts, for which a hybridization probe is available, from 32P-labeled bacteria [Escherichia coli] cells. The RNA is extracted in the absence of **RNase** activity by incorporating an inert, physically removable **RNase** inhibitor throughout the spheroplasting, **cell lysis** and pronase digestion steps. [32P]RNA is separated from [32P]**DNA**, without recourse to phenol extraction or **DNase** treatment, on a Cs2SO4-HCONH2 step gradient in which the precipitated RNA forms a sharp band. Specific transcripts are purified from [32P]RNA by physical separation of the transcript and hybridization probe using gel-exclusion chromatography. The gentleness of this technique enables the co-isolation of **DNA** and can facilitate the analysis of covalently joined RNA-**DNA** replication intermediates.

L5 ANSWER 37 OF 359 CAPLUS COPYRIGHT 2002 ACS
ACCESSION NUMBER: 1975:424840 CAPLUS
DOCUMENT NUMBER: 83:24840
TITLE: Effect of ribonuclease on the association of deoxyribonucleic acid with the membrane in Escherichia coli
AUTHOR(S): McIntosh, Mark A.; Earhart, C. F.
CORPORATE SOURCE: Dep. Microbiol., Univ. Texas, Austin, Tex., USA
SOURCE: J. Bacteriol. (1975), 122(2), 592-8
CODEN: JOBAAY
DOCUMENT TYPE: Journal
LANGUAGE: English

AB The Mg2+-Sarkosyl crystals (M band) procedure was used to study the effect of RNase A on the assocn. of E. coli (DNA) with membrane. Incubation of gently prepd. cell exts. with RNase resulted in the release of DNA from membrane. This effect appears to result from the activation, by RNase, of endonuclease I and subsequent limited activity of this DNase. In support of this explanation, it was demonstrated (i) that the extent of the **RNase**-induced loss of **DNA** from membrane was directly correlated with the endogenous level of endonuclease I, and (ii) that endonucleolytic activity occurred when gently **lysed cell** prepns. were incubated in the presence of **RNase**.

L5 ANSWER 41 OF 359 LIFESCI COPYRIGHT 2002 CSA
ACCESSION NUMBER: 90:26328 LIFESCI
TITLE: A rapid method for the analysis of plasmid content and copy number in various streptomycetes grown on agar plates.
AUTHOR: Labes, G.; Simon, R.; Wohlleben, W.
CORPORATE SOURCE: Lehrstuhl Genet., Fak. Biol., Univ. Bielefeld, Postfach 8640, D-4800 Bielefeld 1, FRG
SOURCE: NUCLEIC ACIDS RES., (1990) vol. 18, no. 8, p. 2197.
DOCUMENT TYPE: Journal
FILE SEGMENT: J; N
LANGUAGE: English
SUMMARY LANGUAGE: English

AB The techniques available for the identification of **plasmid DNA** in gram-positive bacteria such as mycelium-forming streptomycetes are time consuming. Here, we present a new rapid and reproducible method by which the bacteria to be lysed can directly be taken from agar plates. For the **cell lysis** a part of a single colony (1-2 mg) is suspended nearly homogeneously in 20 mu kl TSE solution (50mM Tris, 10mM NaCl, 5mM EDTA pH 8.0) and gently mixed with 20 mu 1 L-solution consisting of 25% sucrose, 3% Ficoll 400, 1 Unit **RNAse** A and 1 mg/ml lysozyme (freshly dissolved) in TB electrophoresis buffer (90mM Tris, 90mM boric acid, 2.5mM EDTA pH 8.2). Immediately 30 mu l of the suspension is filled into the slot of a submerged 0.2% SDS-containing agarose gel. Complete **cell lysis** is achieved by electrophoretic transfer of the negatively charged SDS into the wells for 40 min at 1V/cm. Electrophoresis is continued for 2-4 h at 10V/cm. Before staining with EtBr the gel is rinsed in water to remove SDS.

L5 ANSWER 47 OF 359 MEDLINE
ACCESSION NUMBER: 2001245167 MEDLINE
DOCUMENT NUMBER: 21094320 PubMed ID: 11173096
TITLE: Purification of essentially RNA free plasmid DNA using a modified Escherichia coli host strain expressing ribonuclease A.
AUTHOR: Cooke G D; Cranenburgh R M; Hanak J A; Dunnill P; Thatcher D R; Ward J M
CORPORATE SOURCE: The Advanced Centre For Biochemical Engineering, Department of Biochemical Engineering, University College London, Torrington Place, London WC1E 7JE, UK.
SOURCE: JOURNAL OF BIOTECHNOLOGY, (2001 Feb 23) 85 (3) 297-304. Journal code: AL6; 8411927. ISSN: 0168-1656.
PUB. COUNTRY: Netherlands
Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 200105
ENTRY DATE: Entered STN: 20010517
Last Updated on STN: 20010517
Entered Medline: 20010510

AB Regulatory agencies have stringent requirements for the large-scale production of biotherapeutics. One of the difficulties associated with the manufacture of **plasmid DNA** for gene therapy is the removal of the host cell-related impurity RNA following **cell lysis**. We have constructed a modified Escherichia coli JM107 **plasmid** host (JMRNaseA), containing a bovine pancreatic ribonuclease (**RNaseA**) expression cassette, integrated into the host chromosome at the dif locus. The expressed **RNaseA** is translocated to the periplasm of the cell, and is released during primary **plasmid** extraction by alkaline lysis. The **RNaseA** protein is stable throughout incubation at high pH (approximately 12-12.5), and subsequently acts to hydrolyse host cell RNA present in the neutralised solution following alkaline lysis. Results with this strain harbouring pUC18, and a 2.4 kb pUC18DeltalacO, show that sufficient levels of ribonuclease (**RNase**) activity are produced to hydrolyse the bulk of the host RNA. This provides a suitable methodology for the removal of RNA, whilst avoiding the addition of exogenous animal sourced **RNase** and its associated regulatory requirements.

L5 ANSWER 48 OF 359 WPIDS (C) 2002 THOMSON DERWENT
ACCESSION NUMBER: 1995-358626 [46] WPIDS
DOC. NO. CPI: C1995-156896
TITLE: Recombinant prodn. of reverse transcriptase in RNase-deficient cells - and subsequent purificn. by cation-exchange chromatography to produce enzyme with low levels of contaminants.
DERWENT CLASS: B04 D16
INVENTOR(S): KACIAN, D L; PUTNAM, J G; RIGGS, M G; PUTNAM, J
PATENT ASSIGNEE(S): (GENP-N) GEN-PROBE INC
COUNTRY COUNT: 18
PATENT INFORMATION:

PATENT NO	KIND	DATE	WEEK	LA	PG
WO 9527047	A2	19951012	(199546)*	EN	53
W: AU CA JP KR					
AU 9522371	A	19951023	(199605)		
EP 688870	A1	19951227	(199605)	EN	42
R: AT BE CH DE DK ES FR GB IT LI LU NL SE					
WO 9527047	A3	19951026	(199621)		
JP 09508806	W	19970909	(199746)		74
KR 97702362	A	19970513	(199821)		
AU 696497	B	19980910	(199848)		
US 5935833	A	19990810	(199938)		

US 5998195 A 19991207 (200004)
KR 262258 B1 20000715 (200131)
CA 2186018 C 20020212 (200221) EN

APPLICATION DETAILS:

PATENT NO	KIND		APPLICATION	DATE
WO 9527047	A2		WO 1995-US4092	19950329
AU 9522371	A		AU 1995-22371	19950329
EP 688870	A1		EP 1995-104959	19950403
WO 9527047	A3		WO 1995-US4092	19950329
JP 09508806	W		JP 1995-525899	19950329
			WO 1995-US4092	19950329
KR 97702362	A		WO 1995-US4092	19950329
			KR 1996-705427	19960930
AU 696497	B		AU 1995-22371	19950329
US 5935833	A	Cont of	US 1994-221804	19940401
			US 1997-778217	19970109
US 5998195	A	Cont of	US 1994-221804	19940401
		Cont of	US 1995-443781	19950518
			US 1997-821948	19970321
KR 262258	B1		WO 1995-US4092	19950329
			KR 1996-705427	19960930
CA 2186018	C		CA 1995-2186018	19950329
			WO 1995-US4092	19950329

FILING DETAILS:

PATENT NO	KIND		PATENT NO
AU 9522371	A	Based on	WO 9527047
JP 09508806	W	Based on	WO 9527047
KR 97702362	A	Based on	WO 9527047
AU 696497	B	Previous Publ.	AU 9522371
		Based on	WO 9527047
CA 2186018	C	Based on	WO 9527047

PRIORITY APPLN. INFO: US 1994-221804 19940401; US 1997-778217 19970109; US 1995-443781 19950518; US 1997-821948 19970321

AN 1995-358626 [46] WPIDS

AB WO 9527047 A UPAB: 19951122

A method for producing a polypeptide (A) with RNA-directed and **DNA** -directed **DNA** polymerase activities, comprises: (a) constructing a **plasmid** contg. a gene encoding (A), at least one selectable marker gene, a promoter sequence, and a replicon capable of autonomously replicating the **plasmid** within a suitable host cell; (b) inserting the **plasmid** into a suitable host cell, deficient in the expression of **RNase** activity (pref. E.coli strain 1200) ; (c) growing the host cells contg. the **plasmid** in a liq. culture under conditions capable of promoting cell division and polypeptide gene expression; (d) lysing the host cells; and (e) purifying (A) from the **cell lysate**.

USE - (A) is a reverse transcriptase, useful for commercial applications in amplification reactions.

ADVANTAGE - Recombinant prodn. of the reverse transcriptase in RNAse-deficient host strains and subsequent purificn. results in an enzyme contg. low levels of contaminants that otherwise interfere with transcription-based amplification reactions.

Dwg.0/10

L5 ANSWER 49 OF 359 CAPLUS COPYRIGHT 2002 ACS
ACCESSION NUMBER: 1993:509959 CAPLUS
DOCUMENT NUMBER: 119:109959

TITLE: Rapid isolation and sequencing of double-stranded plasmid DNA
AUTHOR(S): Akella, Rama; Porter, Rebecca
CORPORATE SOURCE: Guthrie Found. Med. Res., Guthrie Clin./Med. Cent., Sayre, PA, 18840, USA
SOURCE: BioTechniques (1993), 14(5), 726, 728, 730
CODEN: BTNQDO; ISSN: 0736-6205
DOCUMENT TYPE: Journal
LANGUAGE: English

AB This report describes a rapid method to sequence unpurified supercoiled plasmid DNA prepd. by a modification of the rapid boiling method described by Holms, D. S. and Quigley, M. (1981). This method eliminates the use of lysozyme for bacterial **cell lysis** and does not require phenol and chloroform extns. or **RNase** digestion prior to the use of the template in **DNA** sequencing reactions. DNA thus made is suitable for restriction anal., if necessary, to verify the presence of an insert of interest in the clone selected before sequencing. Further, co-pptn. of the primer with the denatured template eliminates the extra time required to incubate the template-primer mixt. to anneal the 2 together. The DNA prepd. by this method also permits sequencing gels run with 35S-dATP to be dried immediately after the run without any further manipulations and exposed to the X-ray film without sacrificing any clarity or intensity of the resolved bands. Beginning with overnight cultures, the entire process of verification for presence of an insert in the selected clone up to loading the sequencing gel can be performed in under 4 h using the procedure described here.

L5 ANSWER 58 OF 359 DGENE (C) 2002 THOMSON DERWENT
ACCESSION NUMBER: AAZ32510 DNA DGENE
TITLE: Production of RNA-free cellular components e.g. DNA, proteins, or carbohydrates -
INVENTOR: Hanak J A J; Williams S G
PATENT ASSIGNEE: (COBR-N)COBRA THERAPEUTICS LTD.
PATENT INFO: WO 9953018 A2 19991021 91p
APPLICATION INFO: WO 1999-GB1124 19990413
PRIORITY INFO: GB 1998-7922 19980414
US 1998-81726 19980414
GB 1998-17151 19980806
DOCUMENT TYPE: Patent
LANGUAGE: English
OTHER SOURCE: 1999-620408 [53]

AN AAZ32510 DNA DGENE
AB PCR primers AAZ32510-Z32511 are used to amplify the **RNase** A gene from the expression vector pQR163. The PCR product is used in the production of the pN1RNase A construct. The construct is used in the method of the invention which relates to the production of a substantially RNA-free cellular component. The methods involve culturing a cell producing the cellular component and lysing the cells to produce a **cell lysate**, where the lysate contains the cellular component and sufficient **RNase** activity to degrade substantially all of the RNA molecules present in the lysate. The methods can be used for RNA-free production of cellular components, such as **DNA**, proteins, or carbohydrates, which may have commercial or therapeutic value. RNA is a major contaminant of preparations from **cell lysates** that is difficult to remove as it is similar in size and charge to **DNA**. The invention helps to overcome the limitations of previous methods for RNA removal.

L5 ANSWER 65 OF 359 USPATFULL
ACCESSION NUMBER: 2002:3854 USPATFULL
TITLE: Method for large scale plasmid purification
INVENTOR(S): Lee, Ann L., Lansdale, PA, UNITED STATES
Sagar, Sangeetha, Lansdale, PA, UNITED STATES
PATENT ASSIGNEE(S): Merck & Co., Inc., Rahway, NJ, UNITED STATES, 07065 (U.S. corporation)

	NUMBER	KIND	DATE
PATENT INFORMATION:	US 2002001829	A1	20020103
APPLICATION INFO.:	US 2001-799906	A1	20010306 (9)

RELATED APPLN. INFO.: Division of Ser. No. US 1997-952428, filed on 7 Nov 1997, GRANTED, Pat. No. US 6197553 A 371 of International Ser. No. WO 1996-US7083, filed on 15 May 1996, UNKNOWN Continuation-in-part of Ser. No. US 1995-446118, filed on 19 May 1995, ABANDONED Continuation-in-part of Ser. No. US 1994-275571, filed on 15 Jul 1994, ABANDONED
DOCUMENT TYPE: Utility
FILE SEGMENT: APPLICATION
LEGAL REPRESENTATIVE: MERCK AND CO INC, P O BOX 2000, RAHWAY, NJ, 070650907
NUMBER OF CLAIMS: 11
EXEMPLARY CLAIM: 1
NUMBER OF DRAWINGS: 9 Drawing Page(s)
LINE COUNT: 597
CAS INDEXING IS AVAILABLE FOR THIS PATENT.

AB A process is disclosed for the large scale isolation and purification of plasmid DNA from large scale microbial fermentations. All three forms of plasmid DNA; supercoil (form I), nicked or relaxed circle (form II), and linearized (form III), are individually isolatable using the disclosed process. Highly purified DNA suitable for inclusion in a pharmaceutical composition is provided by the disclosed process.

CAS INDEXING IS AVAILABLE FOR THIS PATENT.

L5 ANSWER 93 OF 359 USPATFULL
ACCESSION NUMBER: 1998:86053 USPATFULL
TITLE: Method for purifying nucleic acids from heterogenous mixtures
INVENTOR(S): Gonzalez, Diana, Placentia, CA, United States
PATENT ASSIGNEE(S): Beckman Instruments, Inc., Fullerton, CA, United States (U.S. corporation)

	NUMBER	KIND	DATE
PATENT INFORMATION:	US 5783686		19980721
APPLICATION INFO.:	US 1995-529148		19950915 (8)

DOCUMENT TYPE: Utility
FILE SEGMENT: Granted
PRIMARY EXAMINER: Wilson, James O.
LEGAL REPRESENTATIVE: May, William H., Harder, P. R.Fulbright & Jaworski
NUMBER OF CLAIMS: 16
EXEMPLARY CLAIM: 1
LINE COUNT: 641
CAS INDEXING IS AVAILABLE FOR THIS PATENT.

AB Discloses DNA isolation and purification methods which involve novel washing steps. The disclosed methods provide a means for isolating and purifying DNA from a homogeneous mixture of DNA of other cellular contaminants by treating silica with the homogeneous mixture containing DNA in the presence of a chaotropic salt solution and then washing and separating the washed and treated silica in successive wash steps with aqueous alcohol wash solutions. A first wash step involves washing the treated silica with a first wash solution of at least 95 wt % alcohol in water. A second wash step similarly involves washing the treated and washed silica with second wash solution of less than 95 wt % alcohol in water.

CAS INDEXING IS AVAILABLE FOR THIS PATENT.

L5 ANSWER 116 OF 359 WPIDS (C) 2002 THOMSON DERWENT
ACCESSION NUMBER: 1999-620408 [53] WPIDS

DOC. NO. CPI: C1999-181161
TITLE: Production of RNA-free cellular components e.g. DNA, proteins, or carbohydrates.
DERWENT CLASS: B04 D16
INVENTOR(S): HANAK, J A J; WILLIAMS, S G
PATENT ASSIGNEE(S): (COBR-N) COBRA THERAPEUTICS LTD
COUNTRY COUNT: 87
PATENT INFORMATION:

PATENT NO	KIND	DATE	WEEK	LA	PG
WO 9953018	A2	19991021	(199953)*	EN	91
RW: AT BE CH CY DE DK EA ES FI FR GB GH GM GR IE IT KE LS LU MC MW NL OA PT SD SE SL SZ UG ZW					
W: AE AL AM AT AU AZ BA BB BG BR BY CA CH CN CU CZ DE DK EE ES FI GB GD GE GH GM HR HU ID IL IN IS JP KE KG KP KR KZ LC LK LR LS LT LU LV MD MG MK MN MW MX NO NZ PL PT RO RU SD SE SG SI SK SL TJ TM TR TT UA UG US UZ VN YU ZA ZW					
AU 9934348	A	19991101	(200013)		
EP 1080179	A2	20010307	(200114)	EN	
R: AT BE CH CY DE DK ES FI FR GB GR IE IT LI LU MC NL PT SE					

APPLICATION DETAILS:

PATENT NO	KIND	APPLICATION	DATE
WO 9953018	A2	WO 1999-GB1124	19990413
AU 9934348	A	AU 1999-34348	19990413
EP 1080179	A2	EP 1999-915927	19990413
		WO 1999-GB1124	19990413

FILING DETAILS:

PATENT NO	KIND		PATENT NO
AU 9934348	A	Based on	WO 9953018
EP 1080179	A2	Based on	WO 9953018

PRIORITY APPLN. INFO: GB 1998-17151 19980806; GB 1998-7922 19980414; US 1998-81726P 19980414

AN 1999-620408 [53] WPIDS

AB WO 9953018 A UPAB: 19991215
NOVELTY - Novel methods are described for producing a substantially RNA-free cellular component.

DETAILED DESCRIPTION - A method of preparing a substantially RNA-free cellular component is new, and comprises culturing cell producing the cellular component (A) in a medium, and lysing the cells to produce a cell lysate, wherein the cell lysate contains (A) and sufficient RNase activity to degrade substantially all of the RNA molecules present in the lysate.

INDEPENDENT CLAIMS are also included for the following:

(1) a method of preparing a substantially RNA-free (A), comprising culturing and lysing cells producing (A) and cells producing RNase in an amount sufficient to degrade substantially all of the RNA present in the preparation;

(2) a host cell that produces a recombinant DNA, a recombinant protein, or a recombinant carbohydrate, and also produces an RNase in a regulated manner;

(3) a composition comprising the host cell of (2) or

(4) a pharmaceutical composition comprising (A) that is substantially RNA-free, in a pharmaceutically acceptable carrier.

USE - The methods can be used for RNA-free production of cellular components, such as DNA, proteins, or carbohydrates, which may have commercial or therapeutic value.

ADVANTAGE - RNA is a major contaminant of preparations from **cell lysates** that is difficult to remove as it is

similar in size and charge to **DNA**. Prior art methods used exogenously produced **RNase** to remove this RNA. However, there are limitations to using exogenously produced **RNase**, in that it is difficult to purify in large amounts and so is expensive to produce. The invention provides methods for RNA-free purification of cellular components that overcome these limitations.
Dwg.0/19

L5 ANSWER 139 OF 359 USPATFULL
ACCESSION NUMBER: 2002:55160 USPATFULL
TITLE: Process for the preparation of endotoxin-free or endotoxin-depleted nucleic acids and/or oligonucleotides for gene therapy
INVENTOR(S): Colpan, Metin, Essen, GERMANY, FEDERAL REPUBLIC OF
Schorr, Joachim, Dusseldorf, GERMANY, FEDERAL REPUBLIC OF
Moritz, Peter, Kerpen, GERMANY, FEDERAL REPUBLIC OF
PATENT ASSIGNEE(S): QIAGEN GMBH

	NUMBER	KIND	DATE
PATENT INFORMATION:	US 2002032324	A1	20020314
APPLICATION INFO.:	US 2001-962459	A1	20010926 (9)

RELATED APPLN. INFO.: Division of Ser. No. US 1999-253702, filed on 22 Feb 1999, GRANTED, Pat. No. US 6297371 Division of Ser. No. US 1996-687529, filed on 18 Oct 1996, GRANTED, Pat. No. US 5990301 A 371 of International Ser. No. WO 1995-EP389, filed on 3 Feb 1995, UNKNOWN

	NUMBER	DATE
PRIORITY INFORMATION:	DE 1994-4403692	19940207
	DE 1994-4422291	19940625
	DE 1994-4431125	19940901
	DE 1994-4432654	19940914

DOCUMENT TYPE: Utility
FILE SEGMENT: APPLICATION
LEGAL REPRESENTATIVE: JACOBSON HOLMAN PLLC, 400 SEVENTH STREET N.W., SUITE 600, WASHINGTON, DC, 20004
NUMBER OF CLAIMS: 17
EXEMPLARY CLAIM: 1
LINE COUNT: 702
CAS INDEXING IS AVAILABLE FOR THIS PATENT.
AB A process for the isolation and purification of nucleic acids and/or oligonucleotides for use in gene therapy wherein said nucleic acids and/or oligonucleotides are isolated or purified from an essentially biological source, characterized in that

said essentially biological sources are lysed, the fractions obtained are optionally freed or depleted from the remainder of said biological sources by per se known mechanical methods, such as centrifugation, filtration;

the fractions thus treated are subsequently treated with affinity chromatographic material or with inorganic chromatographic material for the removal of endotoxins; followed by

isolation of said nucleic acids and/or oligonucleotides on an anion exchanger which is designed such that DNA begins to desorb from the anion exchanger only at an ionic strength corresponding to a sodium chloride solution of a concentration higher by at least 100 mM than one corresponding to the ionic strength at which RNA begins to desorb from the anion exchanger material.

CAS INDEXING IS AVAILABLE FOR THIS PATENT.

L5 ANSWER 141 OF 359 USPATFULL
ACCESSION NUMBER: 1999:151401 USPATFULL
TITLE: Process for the separation and purification of nucleic acids from biological sources
INVENTOR(S): Colpan, Metin, Essen, Germany, Federal Republic of
Schorr, Joachim, Dusseldorf, Germany, Federal Republic of
Moritz, Peter, Kerpen, Germany, Federal Republic of
PATENT ASSIGNEE(S): Qiagen GmbH, Hilden, Germany, Federal Republic of (non-U.S. corporation)

	NUMBER	KIND	DATE	
PATENT INFORMATION:	US 5990301		19991123	
	WO 9521177		19950810	
APPLICATION INFO.:	US 1996-687529		19961018	(8)
	WO 1995-EP389		19950203	
			19961018	PCT 371 date
			19961018	PCT 102(e) date

	NUMBER	DATE
PRIORITY INFORMATION:	DE 1994-4403692	19940207
	DE 1994-4422291	19940625
	DE 1994-4431125	19940914

DOCUMENT TYPE: Utility
FILE SEGMENT: Granted
PRIMARY EXAMINER: Wilson, James O.
LEGAL REPRESENTATIVE: Jacobson, Price, Holman & Stern, PLLC
NUMBER OF CLAIMS: 17
EXEMPLARY CLAIM: 1
LINE COUNT: 681
CAS INDEXING IS AVAILABLE FOR THIS PATENT.
AB A process for the isolation and purification of nucleic acids and/or oligonucleotides for use in gene therapy wherein nucleic acids and/or oligonucleotides are isolated or purified from an essentially biological source, characterized in that

essentially biological sources are lysed, the fractions obtained are optionally freed or depleted from the remainder of biological sources by per se known mechanical methods, such as centrifugation, filtration;

the fractions thus treated are subsequently treated with affinity chromatographic material or with inorganic chromatographic material for the removal of endotoxins; followed by

isolation of nucleic acids and/or oligonucleotides on an anion exchanger which is designed such that DNA begins to desorb from the anion exchanger only at an ionic strength corresponding to a sodium chloride solution of a concentration higher by at least 100 mM than one corresponding to the ionic strength at which RNA begins to desorb from the anion exchanger material.

CAS INDEXING IS AVAILABLE FOR THIS PATENT.

L5 ANSWER 237 OF 359 USPATFULL
ACCESSION NUMBER: 2002:1067 USPATFULL
TITLE: Release of intracellular material and the production therefrom of single stranded nucleic acid
INVENTOR(S): Martin, Sophie E.V., Cambridge, UNITED KINGDOM
Bergmann, Karin, Cambridge, UNITED KINGDOM
Pollard-Knight, Denise V., London, UNITED KINGDOM
PATENT ASSIGNEE(S): Scientific Generics Limited, Cambridge, UNITED KINGDOM (non-U.S. corporation)

	NUMBER	KIND	DATE
PATENT INFORMATION:	US 6335161	B1	20020101
APPLICATION INFO.:	US 1998-30028		19980225 (9)

RELATED APPLN. INFO.: Continuation of Ser. No. WO 1995-GB2024, filed on 25 Aug 1995
DOCUMENT TYPE: Utility
FILE SEGMENT: GRANTED
PRIMARY EXAMINER: Horlick, Kenneth R.
ASSISTANT EXAMINER: Tung, Joyce
LEGAL REPRESENTATIVE: Pillsbury Winthrop LLP
NUMBER OF CLAIMS: 6
EXEMPLARY CLAIM: 1
NUMBER OF DRAWINGS: 5 Drawing Figure(s); 4 Drawing Page(s)
LINE COUNT: 586
CAS INDEXING IS AVAILABLE FOR THIS PATENT.

AB Intracellular material is released from bacterial, yeast, plant, animal, insect or human cells by the application of a low voltage such as 1 to 10 V to a suspension containing the cells. The conditions may be selected such that DNA released from the cells is electrochemically denatured so as to be available for use in an amplification procedure.

CAS INDEXING IS AVAILABLE FOR THIS PATENT.

=> d his

(FILE 'HOME' ENTERED AT 16:23:35 ON 06 JUN 2002)

INDEX 'ADISALERTS, ADISINSIGHT, ADISNEWS, AGRICOLA, ANABSTR, AQUASCI, BIOBUSINESS, BIOCOMMERCE, BIOSIS, BIOTECHABS, BIOTECHDS, BIOTECHNO, CABA, CANCERLIT, CAPLUS, CEABA-VTB, CEN, CIN, CONFSCI, CROPB, CROPU, DDFB, DDFU, DGENE, DRUGB, DRUGLAUNCH, DRUGMONOG2, ...' ENTERED AT 16:24:09 ON 06 JUN 2002

SEA RNAS? AND (DNA? OR PLASMI?)

8	FILE ADISALERTS
9	FILE ADISINSIGHT
844	FILE AGRICOLA
19	FILE ANABSTR
231	FILE AQUASCI
108	FILE BIOBUSINESS
43	FILE BIOCOMMERCE
10723	FILE BIOSIS
463	FILE BIOTECHABS
463	FILE BIOTECHDS
6946	FILE BIOTECHNO
1779	FILE CABA
4020	FILE CANCERLIT
14914	FILE CAPLUS
103	FILE CEABA-VTB
32	FILE CEN
22	FILE CIN
19	FILE CONFSCI
2	FILE CROPB
25	FILE CROPU
4	FILE DDFB
123	FILE DDFU
39403	FILE DGENE
4	FILE DRUGB
7	FILE DRUGNL
260	FILE DRUGU
6	FILE DRUGUPDATES
44	FILE EMBAL

```
             8355   FILE EMBASE
             3511   FILE ESBIOBASE
              548   FILE FEDRIP
                9   FILE FROSTI
               68   FILE FSTA
           195265   FILE GENBANK
              463   FILE IFIPAT
              524   FILE JICST-EPLUS
                3   FILE KOSMET
             4474   FILE LIFESCI
            11798   FILE MEDLINE
               33   FILE NIOSHTIC
               99   FILE NTIS
               38   FILE OCEAN
             1644   FILE PASCAL
               16   FILE PHAR
                1   FILE PHIC
               18   FILE PHIN
              265   FILE PROMT
             8607   FILE SCISEARCH
             3279   FILE TOXCENTER
            14388   FILE USPATFULL
               52   FILE USPAT2
              590   FILE WPIDS
              590   FILE WPINDEX
                8   FILE NAPRALERT
              429   FILE NLDB
L1              QUE RNAS? AND (DNA? OR PLASMI?)
                ---------

     FILE 'GENBANK, DGENE, CAPLUS, USPATFULL, MEDLINE, BIOSIS, SCISEARCH,
     EMBASE, BIOTECHNO, LIFESCI, CANCERLIT, ESBIOBASE, TOXCENTER, CABA,
     PASCAL, AGRICOLA, WPIDS, FEDRIP, JICST-EPLUS' ENTERED AT 16:26:32 ON 06
     JUN 2002
L2         290661 S RNAS? (S) (DNA? OR PLASMI?)
L3            583 S L2 (S) (CELL? (A) LYS?)
L4            359 DUP REM L3 (224 DUPLICATES REMOVED)
L5            359 FOCUS L4 1-

=> s rnas? (s) (clon? or express?) (s) (bacteri? or coli?)
   1 FILES SEARCHED...
   3 FILES SEARCHED...
   8 FILES SEARCHED...
  11 FILES SEARCHED...
  15 FILES SEARCHED...
PROXIMITY OPERATOR LEVEL NOT CONSISTENT WITH
FIELD CODE - 'AND' OPERATOR ASSUMED 'RNAS? (S) '
PROXIMITY OPERATOR LEVEL NOT CONSISTENT WITH
FIELD CODE - 'AND' OPERATOR ASSUMED 'EXPRESS?) (S) '
L6         87836 RNAS? (S) (CLON? OR EXPRESS?) (S) (BACTERI? OR COLI?)

=> s l6 (s) (gen?)
   1 FILES SEARCHED...
   2 FILES SEARCHED...
   5 FILES SEARCHED...
   7 FILES SEARCHED...
   9 FILES SEARCHED...
  11 FILES SEARCHED...
  12 FILES SEARCHED...
  15 FILES SEARCHED...
  17 FILES SEARCHED...
PROXIMITY OPERATOR LEVEL NOT CONSISTENT WITH
FIELD CODE - 'AND' OPERATOR ASSUMED 'L61 (S) '
L7         59604 L6 (S) (GEN?)
```

```
<------User Break------>
u
SEARCH ENDED BY USER

=> s l7 (s) secret? (s) periplasm?
PROXIMITY OPERATOR LEVEL NOT CONSISTENT WITH
FIELD CODE - 'AND' OPERATOR ASSUMED 'L81 (S) SECRET?'
PROXIMITY OPERATOR LEVEL NOT CONSISTENT WITH
FIELD CODE - 'AND' OPERATOR ASSUMED 'SECRET? (S) PERIPLASM'
  18 FILES SEARCHED...
L8          23 L7 (S) SECRET? (S) PERIPLASM?

=> dup rem l8
DUPLICATE IS NOT AVAILABLE IN 'GENBANK, DGENE, FEDRIP'.
ANSWERS FROM THESE FILES WILL BE CONSIDERED UNIQUE
PROCESSING COMPLETED FOR L8
L9           11 DUP REM L8 (12 DUPLICATES REMOVED)

=> d ti l9 1-11

L9   ANSWER 1 OF 11  USPATFULL
TI     Ligand for vascular endothelial growth factor receptor

L9   ANSWER 2 OF 11  USPATFULL
TI     ENTEROCOCCUS FAECALIS POLYNUCLEOTIDES AND POLYPEPTIDES

L9   ANSWER 3 OF 11  USPATFULL
TI     Production and use of recombinant protein multimers with increased
       biological activity

L9   ANSWER 4 OF 11  USPATFULL
TI     DNA encoding erythropoietin multimers having modified 5' and 3'
       sequences and its use to prepare EPO therapeutics

L9   ANSWER 5 OF 11  USPATFULL
TI     Expression of functional antibody fragments

L9   ANSWER 6 OF 11     MEDLINE                          DUPLICATE 1
TI   Relatedness of a periplasmic, broad-specificity RNase from Aeromonas
     hydrophila to RNase I of Escherichia coli and to a family of eukaryotic
     RNases.

L9   ANSWER 7 OF 11  CAPLUS  COPYRIGHT 2002 ACS        DUPLICATE 2
TI   Secretion of recombinant ribonuclease T1 into the periplasmic space of
     Escherichia coli with the aid of the signal peptide of alkaline
     phosphatase

L9   ANSWER 8 OF 11     MEDLINE                          DUPLICATE 3
TI   Expression of Bacillus amyloliquefaciens extracellular ribonuclease
     (barnase) in Escherichia coli following an inactivating mutation.

L9   ANSWER 9 OF 11       GENBANK.RTM.  COPYRIGHT 2002

   TITLE (TI):          Complete DNA sequence of a serogroup A strain of
                        Neisseria meningitidis Z2491
   TITLE (TI):          Direct Submission

L9   ANSWER 10 OF 11      GENBANK.RTM.  COPYRIGHT 2002

   TITLE (TI):          Complete DNA sequence of a serogroup A strain of
                        Neisseria meningitidis Z2491
   TITLE (TI):          Direct Submission

L9   ANSWER 11 OF 11      GENBANK.RTM.  COPYRIGHT 2002
```

TITLE (TI): The genome sequence of the food-borne pathogen Campylobacter jejuni reveals hypervariable sequences
TITLE (TI): Direct Submission

=> d l9 6 7 8 ibib abs

L9 ANSWER 6 OF 11 MEDLINE DUPLICATE 1
ACCESSION NUMBER: 93285983 MEDLINE
DOCUMENT NUMBER: 93285983 PubMed ID: 7685334
TITLE: Relatedness of a periplasmic, broad-specificity RNase from Aeromonas hydrophila to RNase I of Escherichia coli and to a family of eukaryotic RNases.
AUTHOR: Favre D; Ngai P K; Timmis K N
CORPORATE SOURCE: Departement de Biochimie Medicale, Centre Medical Universitaire, Geneva, Switzerland.
SOURCE: JOURNAL OF BACTERIOLOGY, (1993 Jun) 175 (12) 3710-22. Journal code: HH3; 2985120R. ISSN: 0021-9193.
PUB. COUNTRY: United States
Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
OTHER SOURCE: GENBANK-X67054
ENTRY MONTH: 199307
ENTRY DATE: Entered STN: 19930723
Last Updated on STN: 19970203
Entered Medline: 19930713

AB The isolation, sequencing, and characterization of a **periplasmic RNase gene** from Aeromonas hydrophila AH1133 is described. Following subcloning of the **gene** on a 2.7-kb PstI fragment, its direction of transcription and approximate location were determined. Analysis of the nucleotide sequence reveals that the **gene** is 645 bp long, coding for 215 amino acid residues with a total molecular weight of 24,215. A typical leader sequence is present at the beginning of the corresponding protein. Computer analysis revealed strong local similarities to Escherichia **coli RNase** I and to the active site of a family of eukaryotic **RNases**. **Expression** studies indicate that the **RNase** natural promoter functions poorly in E. **coli**. In this organism, the enzyme is mainly localized in the cytoplasm and **periplasm**, although high levels of **expression** lead to significant release into the extracellular medium. Functional and physical characterizations further indicate that the **periplasmic** and cytoplasmic enzymes of A. hydrophila are likely to be the counterparts of E. **coli RNase** I and its cytoplasmic form **RNase** I*: as for the E. **coli** enzymes, the A. hydrophila **RNase** forms have similar sizes and show broad specificity, and the **periplasmic** form is more active towards natural polymer RNA than its cytoplasmic counterpart. Both forms are relatively thermosensitive and are reversibly inactivated by up to 0.6% sodium dodecyl sulfate. Southern hybridization revealed homology to E. **coli** K-12 and Shigella sp. **genomic** DNA, a finding which correlates with the presence of **secreted RNases** in these organisms. In contrast, species of phylogenetically closer **genera**, such as Vibrio and Plesiomonas, did not hybridize to the A. hydrophila **RNase gene**.

L9 ANSWER 7 OF 11 CAPLUS COPYRIGHT 2002 ACS DUPLICATE 2
ACCESSION NUMBER: 1990:606105 CAPLUS
DOCUMENT NUMBER: 113:206105
TITLE: Secretion of recombinant ribonuclease T1 into the periplasmic space of Escherichia coli with the aid of the signal peptide of alkaline phosphatase
AUTHOR(S): Fujimura, Takao; Tanaka, Toshiki; Ohara, Kanako; Morioka, Hiroshi; Uesugi, Seiichi; Ikehara, Morio; Nishikawa, Satoshi

CORPORATE SOURCE: Fac. Pharm. Sci., Osaka Univ., Suita, 565, Japan
SOURCE: FEBS Lett. (1990), 265(1-2), 71-4
CODEN: FEBLAL; ISSN: 0014-5793
DOCUMENT TYPE: Journal
LANGUAGE: English

AB The RNase T1 gene of Aspergillus oryzae was ligated to a synthetic gene for the signal peptide of E. coli alk. phosphatase. When this fusion **gene** was **expressed** in E. **coli** under the control of the trp promoter, active **RNase** T1 having the correct N-terminal sequence was **secreted** into the **periplasmic** space, indicating that the heterologous signal peptide had been cleaved off correctly. The enzyme could be readily purified from the periplasmic fraction with a yield of 1.8 mg from 1 L culture. Adopting the same strategy, it was possible to produce a labile mutant of RNase T1 (Glu-58 .fwdarw. Ala mutant) in E. coli, the yield of the purified mutant enzyme being 2.0 mg from 1 L culture.

L9 ANSWER 8 OF 11 MEDLINE DUPLICATE 3
ACCESSION NUMBER: 87248102 MEDLINE
DOCUMENT NUMBER: 87248102 PubMed ID: 3297926
TITLE: Expression of Bacillus amyloliquefaciens extracellular ribonuclease (barnase) in Escherichia coli following an inactivating mutation.
AUTHOR: Paddon C J; Hartley R W
SOURCE: GENE, (1987) 53 (1) 11-9.
Journal code: FOP; 7706761. ISSN: 0378-1119.
PUB. COUNTRY: Netherlands
Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 198708
ENTRY DATE: Entered STN: 19900305
Last Updated on STN: 19900305
Entered Medline: 19870805

AB An inactivated **gene** for Bacillus amyloliquefaciens extracellular ribonuclease (barnase) has previously been **cloned** and sequenced following transposon mutagenesis. The intact **gene** could not be assembled in Escherichia **coli** and is presumed to be lethal. Therefore, we introduced specific mutations into the barnase **gene** to prevent its lethal effect. A Gln-73 mutant **gene** was stable in E. **coli** but only produced low amounts of barnase antigen. Mutants containing Asp, Gln or Arg, instead of His-102, at the active site were identified by immunological screening for barnase antigen. None of the mutant proteins with alterations at aa residue 102 possessed **RNase** activity. The level of barnase (Asp-102) was higher in E. **coli** than in B. subtilis but the protein was not processed to the correct size in E. **coli**. To obtain correct processing, the barnase (Asp-102) structural **gene** was fused to the E. **coli** alkaline phosphatase promoter and signal sequence (phoA). Cells containing this construct **secreted** correctly processed barnase (Asp-102) into the **periplasmic** space and culture supernatant at a level of 20 mg/l. Barnase (Asp-102) was purified and found to have an identical N-terminus and a thermal unfolding curve that was nearly identical to that of active barnase (His-102). The **cloning** and **expression** of barnase in E. **coli** will allow detailed analysis of barnase protein folding by molecular **genetic** approaches.

=> d his

(FILE 'HOME' ENTERED AT 16:23:35 ON 06 JUN 2002)

INDEX 'ADISALERTS, ADISINSIGHT, ADISNEWS, AGRICOLA, ANABSTR, AQUASCI, BIOBUSINESS, BIOCOMMERCE, BIOSIS, BIOTECHABS, BIOTECHDS, BIOTECHNO, CABA,

CANCERLIT, CAPLUS, CEABA-VTB, CEN, CIN, CONFSCI, CROPB, CROPU, DDFB, DDFU, DGENE, DRUGB, DRUGLAUNCH, DRUGMONOG2, ...' ENTERED AT 16:24:09 ON 06 JUN 2002

SEA RNAS? AND (DNA? OR PLASMI?)

8	FILE ADISALERTS
9	FILE ADISINSIGHT
844	FILE AGRICOLA
19	FILE ANABSTR
231	FILE AQUASCI
108	FILE BIOBUSINESS
43	FILE BIOCOMMERCE
10723	FILE BIOSIS
463	FILE BIOTECHABS
463	FILE BIOTECHDS
6946	FILE BIOTECHNO
1779	FILE CABA
4020	FILE CANCERLIT
14914	FILE CAPLUS
103	FILE CEABA-VTB
32	FILE CEN
22	FILE CIN
19	FILE CONFSCI
2	FILE CROPB
25	FILE CROPU
4	FILE DDFB
123	FILE DDFU
39403	FILE DGENE
4	FILE DRUGB
7	FILE DRUGNL
260	FILE DRUGU
6	FILE DRUGUPDATES
44	FILE EMBAL
8355	FILE EMBASE
3511	FILE ESBIOBASE
548	FILE FEDRIP
9	FILE FROSTI
68	FILE FSTA
195265	FILE GENBANK
463	FILE IFIPAT
524	FILE JICST-EPLUS
3	FILE KOSMET
4474	FILE LIFESCI
11798	FILE MEDLINE
33	FILE NIOSHTIC
99	FILE NTIS
38	FILE OCEAN
1644	FILE PASCAL
16	FILE PHAR
1	FILE PHIC
18	FILE PHIN
265	FILE PROMT
8607	FILE SCISEARCH
3279	FILE TOXCENTER
14388	FILE USPATFULL
52	FILE USPAT2
590	FILE WPIDS
590	FILE WPINDEX
8	FILE NAPRALERT
429	FILE NLDB

L1 QUE RNAS? AND (DNA? OR PLASMI?)

FILE 'GENBANK, DGENE, CAPLUS, USPATFULL, MEDLINE, BIOSIS, SCISEARCH, EMBASE, BIOTECHNO, LIFESCI, CANCERLIT, ESBIOBASE, TOXCENTER, CABA,

PASCAL, AGRICOLA, WPIDS, FEDRIP, JICST-EPLUS' ENTERED AT 16:26:32 ON 06 JUN 2002

```
L2        290661 S RNAS? (S) (DNA? OR PLASMI?)
L3           583 S L2 (S) (CELL? (A) LYS?)
L4           359 DUP REM L3 (224 DUPLICATES REMOVED)
L5           359 FOCUS L4 1-
L6         87836 S RNAS? (S) (CLON? OR EXPRESS?) (S) (BACTERI? OR COLI?)
L7         59604 S L6 (S) (GEN?)
L8            23 S L7 (S) SECRET? (S) PERIPLASM?
L9            11 DUP REM L8 (12 DUPLICATES REMOVED)

=> log h
COST IN U.S. DOLLARS                                SINCE FILE      TOTAL
                                                      ENTRY    SESSION
FULL ESTIMATED COST                                  169.63     171.96

DISCOUNT AMOUNTS (FOR QUALIFYING ACCOUNTS)          SINCE FILE      TOTAL
                                                      ENTRY    SESSION
CA SUBSCRIBER PRICE                                   -2.48      -2.48

 SESSION WILL BE HELD FOR 60 MINUTES
STN INTERNATIONAL SESSION SUSPENDED AT 17:12:28 ON 06 JUN 2002
```